Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 40-43, 45-56 and 58-60 are pending in the application, with claim 40 being the independent claim. Claims 44 and 57 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Applicants request entry of the following Amendment in view of the Examiner's suggestions. Claims 40 and 46 have been amended so that the claims recite either a tPA or K2S molecule and do not recite a variant of tPA or K2S. Support for amended claims 40 and 46 can be found in the originally filed claims and *inter alia*, at [0030] - [0032] in the specification. Claim 51 has been amended to further include a washing step, as suggested by the Examiner. Support for amended claim 51 can be found at [0052] in the specification. These changes are believed to introduce no new matter and are believed to place the application in condition for allowance. Thus, entry of the foregoing amendment is respectfully requested.

Based on the above Amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objection to the Specification

The Examiner has objected to the specification under 35 U.S.C § 112, first paragraph as allegedly lacking sufficient written description for enablement. Paper No. 042604, page 2. Applicants respectfully disagree. Applicants note that according to the requirements for the deposit of biological material, the biological material need not be

deposited, *inter alia*, if it can be made or isolated without undue experimentation. 37 C.F.R. § 1.802(b) (2003). Based on the description of pComb3HSS in the specification and based on guidance in the art, Applicants assert that the specification contains adequate written description. Nevertheless, in order to expedite prosecution, Applicants have cancelled claim 57, rendering the objection moot.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claim 57 under 35 U.S.C. § 112, first paragraph as allegedly lacking sufficient written description for enablement. Paper No. 042604, page 2. Applicants have cancelled claim 57, rendering the rejection moot.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 1-15, 17-24 and 31-37 under 35 U.S.C. § 112, second paragraph. Paper No. 042604, page 2. Applicants note that claims 1-39 were previously cancelled in the Amendment and Reply filed on February 4, 2004. Thus, the Examiner's rejections with respect to claims 1-15, 17-24 and 31-37 are rendered moot.

The Examiner has rejected claim 40 has allegedly being indefinite with regard to the phrases "a tPA variant" and "a K2S variant." Paper No. 042604, page 2. Applicants note that variants of tPA and K2S are specifically referred to and described in the specification. *See*, e.g., Specification, pages 10-11, paragraphs 39-41; page 15, paragraph 54; pages 16-17, paragraphs 56-58; page 20, paragraph 90; page 31, paragraph 104. Nevertheless, in order to expedite prosecution, Applicants have amended claim 40 so that it no longer recites the phrases "a tPA variant" or "a K2S variant." Accordingly,

Applicants respectfully request that the rejection of claim 40 be reconsidered and withdrawn.

The Examiner has rejected claim 57 as allegedly being indefinite. Paper No. 042604, page 3. Applicants have cancelled claim 57, rendering the rejection moot.

The Examiner has rejected claim 51 as allegedly being indefinite for not indicating wash conditions. Paper No. 042604, page 3. Applicants have amended claim 51 to include wash conditions. Thus, Applicants respectfully request that the rejection of claim 51 be reconsidered and withdrawn.

The Examiner has also included claims 41-43, 52-56 and 56-60 with the rejection under U.S.C. § 112, second paragraph, as they depend from claim 40. Paper No. 042604, page 3. Based on the amendment to claim 40 noted above, the rejection of claims 41-43, 52-56 and 56-60 have been rendered moot.

Finally, the Examiner has rejected claim 44 for allegedly being indefinite. Paper No. 042604, page 3. Applicants have cancelled claim 44, rendering this rejection moot.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claims 40-56 and 58-60 under 35 U.S.C. § 103 as allegedly being obvious over Wong *et al.* (EP 0357391 A2) in view of Obukowicz *et al.*, *Biochemistry 29*:9737-9745 (1990), Niwa *et al.* (U.S. Patent No. 5,840,533) and the nucleic acid encoding human tissue plasminogen activator, for the reasons set forth in the prior Office Action mailed September 12, 2003. Paper No. 042604, page 4.

Applicants respectfully disagree. Applicants assert that the claimed invention is directed to a DNA molecule comprising: (1) tPA or K2S; (2) OmpA *and*; (3) either the

signal peptide sequence SEGN or SEGNSD. Furthermore, the present application provides that, when recombinant tPA and/or K2S molecules are expressed in *a prokaryotic host cell,* they are efficiently secreted into the culture supernatant utilizing the beneficial combination of *both* an OmpA signal peptide *and the SEGN or SEGNSD peptide.* In fact, the specification, at paragraph [0103] notes that when recombinant K2S is expressed in *E. coli* cells, 68% of the recombinant K2S protein can be directly isolated from the culture supernatant with only 32% of the recombinant K2S secreted into the periplasm. One important embodiment of the claimed invention is that the inclusion of the SEGN or SEGNSD sequence facilitates secretion and also results in a larger than expected fraction of the recombinant protein to be secreted into the culture supernatant.

The references that the Examiner cites do not teach or suggest the inclusion of a SEGN or SEGNSD peptide within a fusion construct in order that the SEGN or SEGNSD peptide facilitates secretion of a recombinant protein to such an extent that, for example, 68% of the protein can be isolated directly from the culture supernatant.

The Examiner states that the '533 patent (Niwa *et al.*) teaches a longer half-life time of the K2S variants and greater thrombolytic activity. Paper No. 042604, page 3. Furthermore, the Examiner alleges that the '533 patent suggests the expression of the K2S variants in *E. coli* fused to a signal peptide for translocation. Paper No. 042604, page 4. The Examiner also alleges that it would have been obvious to one of ordinary skill in the art at the time of the invention to attach the coding sequence OmpA to the nucleic acid sequence of SEQ ID NO:1 of the '533 patent.

The nucleic acid sequence of SEQ ID NO:1 of the '533 patent does not contain the sequence SEGN or SEGNSD. Additionally, the '533 patent only discusses the use of

a signal peptide when the host cell is a yeast or animal cell. The '533 patent, col. 4, lines 55-67. In fact, the '533 does not contemplate using an *E. coli* host cell to produce recombinant protein which is secreted into the culture medium.

With regard to using prokaryotic host cells for expression of tPA, the '533 patent states the following:

> [w]hen a bacterium such as *E. coli* is used as a host cell, thus produced new t-PA generally exist in cells of the cultured transformant and the cells are collected by filtration or centrifugation, and cell wall and/or cell membrane thereof are destroyed in a conventional manner . . . to give debris. From the debris, the new t-PA can be purified and isolated . . .

The '533 patent, col. 6, lines 21-27.

Thus, while the '533 patent may disclose variants of human tPA, the studies in the '533 patent only discuss the use of a prokaryotic host cell to isolate protein from within the cell, or from the cell debris, *not* from the culture supernatant. Furthermore, the '533 patent does not contemplate or teach using a signal peptide, such as OmpA, together with SEGN or SEGNSD, in any type of host cell in order that the combination of OmpA and SEGN or SEGNSD facilitates secretion into the culture medium.

Wong *et al.* disclose an expression system in *E. coli* where a heterologous protein is fused in frame to a nucleic acid sequence encoding the OmpA signal peptide. Wong *et al.* do not teach or suggest a fusion construct comprising a sequence encoding the peptide SEGN or SEGNSD, especially with regard to utilizing these sequences to facilitate secretion into the culture supernatant.

Obukowicz *et al.* teach expression of a fusion construct comprising the signal peptide PhoA and the K2S protein. Obukowicz *et al.* also do not teach or suggest a fusion construct comprising a sequence encoding the peptide SEGN or SEGNSD.

Thus Obukowicz *et al.* and/or the '533 patent (Niwa *et al.*) do not rescue the deficiencies of Wong *et al.* Absent a motivation or suggestion to combine reference teachings, the Examiner has improperly relied upon hindsight reasoning in combining references in support of the rejection under 35 U.S.C. § 103(a). *Cf.* M.P.E.P. §§ 2142 and 2143 (rev. 8th ed. Feb. 2003).

Furthermore, the known nucleic acid sequence encoding human tissue plasminogen activator also does not rescue the deficiency of Wong *et al.* as it merely teaches the sequence of human tPA, and does not provide any suggestion for a nucleic acid sequence encoding tPA to be operably linked to the signal sequence OmpA or the sequence encoding either the peptide SEGN or SEGNSD.

Wong *et al.* in view of Obukowicz *et al.*, the '533 patent (Niwa *et al.*) and the nucleic acid encoding human tissue plasminogen activator do not render claims 40-56 and 58-60 obvious as they do not, *inter alia*, teach or suggest all of the limitations of the claims. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 103 be reconsidered and withdrawn.

Provisional Double Patenting Rejection

The Examiner has provisionally rejected claims 40-60 under the judicially created doctrine of obviousness-type double patenting over claims 12-18, 21-29 and 31-53 of copending Appl No. 09/987,457. Paper No. 042604, page 5. Applicants file

herewith a terminal disclaimer under 37 C.F.R. § 1.321(c) to overcome the provisional rejection based on a nonstatutory double patenting ground. The instant application and the conflicting application are commonly owned.

Reconsideration and withdrawal of the provisional rejection are respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

45,986

for Eric K. Steffe
Attorney for Applicant
Registration No. 36,688

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1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

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